Abstract

Transthyretin is one of the three major thyroid hormone-binding proteins in the blood plasma of vertebrates that determine the distribution of thyroid hormones between the extracellular aqueous and the intracellular lipid phase in tissues. During evolution of vertebrates, structure changes of transthyretin are most pronounced in amino acid sequence in the N-terminal region. The N-termini of transthyretin subunits are longer and more hydrophobic in avian, amphibian and fish than in eutherian transthyretins. The evolution of transthyretin function, namely the binding of thyroid hormones, was shown decreased in affinities for triiodothyronine but increased in affinities for thyroxine. In order to study the influences of N-termini on binding affinities of transthyretin to thyroid hormones, normal crocodile transthyretin and two chimeric transthyretins (with N-terminus of *Xenopus* transthyretin and with N-terminus of human transthyretin) were produced in yeast *Pichia pastoris*, using the extracellular expression vectors, pPIC 3.5 and pPIC 9. These recombinant transthyretin proteins showed the correct size of 15 kilodaltons on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western analysis, and self-associated into tetrameric form as determined by size exclusion chromatography. Recombinant normal crocodile transthyretin had similar affinities, for thyroxine and triiodothyronine, to chicken transthyretin. Whereas, both recombinant chimeric transthyretins had reduced affinities, but with different reduction extent. The evidence supported the role of N-terminal region in thyroid hormones binding of transthyretin. Expression of mutant transthyretins in *Pichia pastoris* provides the opportunity to study the structure and function relationships of the N-terminus of transthyretin.